side of a BoNT A cleavage site and a quencher located on the other side of the BoNT A cleavage to the test assay and control assay; incubating the isolated peptide with the beads from the test assay; incubating the isolated peptide with the beads from the control assay; separating the beads from the test assay from a test assay solution comprising the isolated peptide; separating the beads from the control assay from a control assay solution comprising the isolated peptide; measuring fluorescence intensity of the test assay solution; measuring fluorescence intensity of the control assay solution; comparing the fluorescence of the test assay solution and the control assay solution; and determining the concentration of BoNT A in the sample by comparison of fluorescence intensity of the test assay solution with a standard curve prepared using known concentrations of BoNT A Lc. In an embodiment, the method further comprises adding bovine serum albumin to the pH buffering compound. In another embodiment, the method further comprises adding polysorbate 20 present to the pH buffering compound. In an embodiment, the polysorbate 20 is added to a final concentration of 0.05-0.10%.

[0016] An embodiment is a method for measuring the activity of BoNT A comprising incubating SEQ ID NO: 1 with BoNT A to form a sample; injecting the sample onto an HPLC column; preparing a chromatogram of the elution of various components of the sample; analyzing the chromatogram to determine how much of the isolated peptide was cleaved based upon the size of peaks correlating to the isolated peptide and cleaved portions of the isolated peptide.

[0017] An embodiment is a method for identifying a BoNT A inhibitor comprising incubating BoNT A with a potential inhibitor to form a first sample; incubating BoNT A without a potential inhibitor to form a second sample; adding SEQ ID NO: 1 to the first sample; adding SEQ ID NO: 1 to the second sample; stopping the reactions by adding acid to the first and second sample; injecting the first sample onto an HPLC column; preparing a chromatogram of the elution of various components of the first sample; injecting the second sample onto an HPLC column; preparing a chromatogram of the elution of various components of the second sample; analyzing the chromatogram for the first sample and the second sample to determine how much of the isolated peptide was cleaved based upon the size of peaks correlating to the isolated peptide and cleaved portions of the isolated peptide; and determining that a potential inhibitor of BoNT A is a BoNT A inhibitor if the size of the peak correlating to the isolated peptide for the first sample is taller than the size of the peak for the isolated peptide the second sample.

[0018] An embodiment is a method of treating an individual in need of treatment for a disorder due to BoNT A comprising administering a composition comprising SEQ ID NO: 1.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure. The disclosure may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0020] FIG. **1** depicts an HPLC chromatogram illustrating hydrolysis of P6 (SEQ ID NO: 2) catalyzed by BoNT A light chain.

[0021] FIG. **2** depicts a plot of fluorescence values vs. time in the absence (Blank) and presence (A-Lc) of BoNT A light chain. The substrate is flP6 ([DabcylK] (SEQ ID NO: 2) [SFC]).

[0022] FIG. **3**A depicts the effects of BoNT A light chain concentrations on hydrolysis of flP6 ([DabcylK] (SEQ ID NO: 2) [SFC]).

[0023] FIG. **3**B depicts the effects of substrate concentrations on hydrolysis of flP6 ([DabcylK] (SEQ ID NO: 2) [SFC]), catalyzed by BoNT A light chain.

[0024] FIG. **4** depicts initial hydrolysis rates (v) of flP6 ([DabcylK] (SEQ ID NO: 2) [SFC]) at various concentrations, catalyzed by BoNT A light chain.

DETAILED DESCRIPTION

[0025] The disclosure relates to botulinum toxin and substrates thereof. It will be appreciated that for simplicity and clarity of illustration, where considered appropriate, reference numerals may be repeated among the figures to indicate corresponding or analogous elements. In addition, numerous specific details are set forth in order to provide a thorough understanding of the example embodiments described herein. However, it will be understood by those of ordinary skill in the art that the example embodiments described herein may be practiced without these specific details. In other instances, methods, procedures and components have not been described in detail so as not to obscure the embodiments described herein.

[0026] The following definitions and explanations are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following Description or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition. Definitions and/or interpretations should not be incorporated from other patent applications, patents, or publications, related or not, unless specifically stated in this specification or if the incorporation is necessary for maintaining validity.

[0027] The term "FRET substrate", as used herein, refers to a peptide substrate with a fluorophore and quencher placed on opposite sides of the BoNT A cleavage site within the substrate.

[0028] The term "HPLC", as used herein, refers to high performance liquid chromatography. The term HPLC includes RPHPLC (reverse phase high performance liquid chromatography). RPHPLC is a chromatographic method that uses a non-polar stationary phase.

[0029] The term "2,4-diaminobutanoic acid", as used herein, is also referred to as 2,4-diaminobutyric acid and abbreviated as dab or dbu.

[0030] The term "2-aminobutanoic acid", as used herein, is also referred to as 2-aminobutyric acid and abbreviated as B or abu.

[0031] The term "polysorbate-20", as used herein, is also referred to as poly(oxyethylene)$_x$-sorbitane-monolaurate or Tween® 20.